Effects of Fin Clipping for DNA Sampling on Physiological Stress, Swimming, and Survival of Chinook Salmon

Investigators

Donald E. Portz, Ph.D.
Fisheries Biologist
Fisheries and Wildlife Resources Group
Bureau of Reclamation
Denver, CO 80225
dportz@usbr.gov

Zak Sutphin
Fisheries Biologist
Fisheries and Wildlife Resources Group
Bureau of Reclamation
Denver, CO 80225
zsutphin@usbr.gov

Summary

DNA sampling at the Tracy Fish Collection Facility (Central Valley Project) and John E. Skinner Delta Fish Protection Facility (State Water Project) for juvenile Chinook salmon estimate the timing, abundance, and proportion of different races of Chinook salmon leaving the Sacramento-San Joaquin Delta. The advent of DNA typing has substantially improved the capability of identifying distinct Chinook races, compared to the size-at-date criteria method (Johnson *et al.* 1992), that is still in use. Fin clips are used to obtain tissue samples for genetic analysis and are widely used fisheries management for marking individual fish. Genetic data are critically needed to conserve and manage endangered and threatened fishes, and should not directly or indirectly compromise their survival, especially considering their diminished population sizes. It is still undecided in the scientific community if fin clipping causes harm and affects survival. Some data suggest that fin clipping can greatly reduce survival and hinder growth (Saunders and Allen 1967, Shetter 1967, Webber and Wahle 1969, Coble 1971, Nicola and Cordone 1973, O'Grady 1984, Bergstedt 1985, Hansen 1988), and in addition, extensive fin damage caused by tissue sampling can result in compromised survival (O'Grady 1984). Conversely, fin clipping has also been shown to have no effect on survival or growth (Armstrong 1947, Radcliffe 1950, Horak 1969, Gjerde and Refstie 1988, Conover and Sheehan 1999, Pratt and Fox 2002, Vander Haegen *et al.* 2005, Champagne *et al.* 2008). Fin clipping could adversely affect swimming performance, predator avoidance, and the ability to find and capture prey. Handling and severing fins is known to be stressful to fish (Sharpe *et al.* 1998, Barton *et al.* 2002) and provide a potential vector for bacterial infection (Elliot and Pascho 2001, Vander Haegen *et al.* 2005). Decreased survival of fish can result when physiological stress responses remain elevated and become debilitating, leaving fish vulnerable to predation or swimming challenges (Barton 2002, Portz 2007). A few studies have examined the effects of fin clipping on swimming velocity (Radcliffe 1950, Horak 1969, Champagne *et al.* 2008); however to our knowledge no studies involving burst swimming have been performed.

Burst swimming is important in evading predators, catching prey, and danger avoidance (Portz 2007). An assessment of the effects of fin clipping of juvenile Chinook salmon for DNA sampling at the Tracy Fish Collection Facility and John E. Skinner Delta Fish Protective Facility is needed to address whether fin sampling may inadvertently be compromising these fish after release. It is important to conduct an evaluation under on-site conditions so fish would be exposed to the array of natural occurring environmental factors including potential pathogens and water quality.

Problem Statement

Handling, anesthetizing, and taking fin tissue samples of juvenile Chinook salmon for genetic analyses at the Tracy Fish Collection Facility and John E. Skinner Delta Fish Protection Facility may compromise survival. While genetic data are crucial to conserve and manage this species, tissue sampling should not directly or indirectly compromise their survival, especially considering their diminished population sizes. An assessment of the effects of fin clipping is needed to address whether the fin sampling protocol may inadvertently be compromising fish health and survival after release.

Goals and Hypotheses

Goals:

1. Determine if handling, anesthetizing, and fin clipping for DNA samples affect juvenile Chinook salmon physiological stress.

2. Determine if handling, anesthetizing, and fin clipping for DNA samples affect scale loss and external tissue damage in juvenile Chinook salmon.

3. Determine if handling, anesthetizing, and fin clipping for DNA samples affect the burst swimming performance of juvenile Chinook salmon, possibly hindering their ability to avoid predator capture

4. Determine if handling, anesthetizing, and fin clipping for DNA samples affect the short-term survival (168 h) of juvenile Chinook salmon.

Hypotheses:

1. If fin clip tissue sampling is physiological stressful to juvenile Chinook salmon, then fin-clipped fish should have heightened plasma cortisol, glucose, and lactate concentrations compared to unclipped (control) fish and those handled but not clipped.

2. If fin clip tissue sampling affects scale loss and external tissue damage in juvenile Chinook salmon, then fin-clipped fish will have greater areas of skin ulcerations and damage compared to unclipped (control) fish and those handled but not clipped fish.

3. If fin clip tissue sampling affects the burst swimming performance of juvenile Chinook salmon, then maximum swimming velocities of fin-clipped fish will

be slower and maximum C-start angles higher (less bending) compared to unclipped (control) fish and those handled but not clipped.

4. If fin clip tissue sampling affects the short-term survival (168 h) of juvenile Chinook salmon, then fin-clipped fish will have greater mortality compared to unclipped (control) fish and those handled but not clipped fish.

Materials and Methods

Source and Care of Fish

Sacramento River Chinook salmon (*Oncorhynchus tshawytscha*) used in this study will be obtained in March 2010 from the Mokelumne River Hatchery (Clements, CA) or the Coleman National Fish Hatchery (Anderson, California), and transported to the Tracy Fish Collection Facility (Byron, California). Juvenile fall-run Chinook salmon will be maintained in 757-L circular tanks equipped with aerated, well water /Delta water mix. Fish will be held under a natural photoperiod (37° 44' 23" N latitude) with natural and halogen light, and fed Silver Cup salmon feed pellets (Nelson and Son, Inc., Murray, Utah) at 1.5–2% body weight per day. Treatment and control salmon may be marked with implanted, colored microspheres on dorsal and anal fins with a high pressure needle (Photonic tagging; New West Technology, Arcata, California) to consolidate fish when holding 168 h to conserve tank space.

The Experiment: Effects of fin clipping

The experiment will be compromised of three groups of juvenile salmon (*ca*. 110 mm): (1) control, (2) handled but not caudal clipped, and (3) handled/fin caudal clipped fish. The handled/fin clipped fish will undertake the normal tissue sampling protocol of netting, anaesthetizing, handling, excising the upper lobe of the caudal fin, and releasing into one of two holding tank conditions: (1) raw Delta, and (2) ozonated/ultraviolet sterilized water for a 2-h recovery period to simulate their normal post-sampling holding. The same procedures will be performed on the handled-only fish but no tissue sampling will take place. Water quality (*i.e*., temperature, dissolved oxygen concentration, pH) in the holding conditions will be monitored throughout the study. Twelve replicates of each group will be collected each month for April, May, and June.

Physiological Stress Response

A control fish will be captured and removed from previously undisturbed 757-L tanks with modified 10-cm x 18-cm dip nets with a 1.5-L plastic reservoir sewn into the cod-end, so that fish could be transferred in water to minimize stress. All transfers of control fish will be accomplished quickly (<30 s) with minimal disturbance and handling trauma to the fish. Treatment fish will be handled and sampled according to standard tissue sampling protocol used by fish facility personnel. Control and treatment fish will be quickly transferred to a bath containing a lethal dose of tricaine methanesulfate (MS-222, Argent Chemical Laboratories, Inc., Redmond, Washington; 200 mg/L), which immobilizes them in less than 30 s. This anesthetic dose inhibits stress-related increases in plasma cortisol concentration in salmon. Blood will be collected from the severed caudal peduncle in 40-µl, heparinized microhematocrit capillary tubes. Blood samples from the treatment groups under the two holding conditions will be collected at 0, 1, 4,

24, 168 h post-treatment. Weights (±0.01 g) and measurements (TL, ±1 mm) of each fish using an electronic balance and fish measuring board will be recorded. Collected blood will be immediately centrifuged using a microhematocrit centrifuge (Clay-Adams Autocrit Ultra3) for 4 min at 12,000 x g to separate the plasma from the packed cells (Becton Dickinson Diagnostics, Sparks, Maryland). Hematocrit (packed cell volume) will be measured shortly after collection. Plasma obtained with from each fish will be transferred into plastic cryogenic freezing vials and temporarily stored in a 10-L liquid-nitrogen dewar flask (-196 ˚C). These samples will then be shipped to Denver, CO were they will be stored in a -80 ºC freezer for storage for analyses of plasma cortisol, lactate, and glucose once field component is complete. Plasma cortisol concentrations will be measured using a modified enzyme immunoassay (ELISA) at the University of California, Davis Endocrinology Lab, and plasma lactate and glucose will be measured with a polarographic analyzer (YSI 2700 Select, Yellow Springs Incorporated, Yellow Springs, Ohio) in the Fisheries and Wildlife Group's Fish Physiology Lab.

External Tissue Damage

Scale loss and external tissue damage will be determined in the control and the two treatment groups immediately post-treatment and after a 168-h holding period in 190-L tanks using fluorescein (AK-Fluor®, Akorn, Inc., Decatur, Illinois). Fluorescein is a nontoxic fluorescent dye that can be used to rapidly and easily detect scale loss and tissue lesions and ulcers by binding to breaks or tears in the epithelial barrier of soft tissue. Fish will be anesthetized in a MS-222 bath (40 mg/L) and transferred to a solution of 0.20 mg fluorescein/1ml water for 5 min and then rinsed in three separate clean water baths for 2 min. The fish will then be euthanized in a 200 mg/L MS-222 bath and immediately examined for skin damage under an ultraviolet light (Model UVGL-58, Mineralight, Upland, California). Photographs are taken in complete darkness under ultraviolet light using a Nikon D-100 digital camera. Severity of tissue damage will be categorized, external bacterial and fungal infections will be diagnosed, and total damaged area will be quantified. Weights (±0.01 g) and measurements (TL, ±1 mm) of each fish using an electronic balance and fish measuring board will be recorded.

Burst Swimming Performance

A juvenile salmon will be quickly transferred to an acrylic raceway for measuring burst swimming performance (including mean velocity, maximum velocity, mean acceleration, maximum acceleration, and C-start angles). Burst swimming performance will be determined for control at pre-experiment and 168 h, and the two treatment groups at 0 and 168 h for both holding water quality conditions. The burst swimming raceway (220-cm-long with a 30-cm-wide swimming channel) will be filled to 25-cm depth to minimize vertical swimming. Startle responses and burst swimming speeds will be filmed with a Phantom v4.2 high-speed camera (Vision Research, Inc., Wayne, New Jersey) fitted with a wide angle lens and lighted by four, 150-W floodlights situated 1.3 m above the raceway. Fish will be stimulated to swim with a tethered tennis ball that strikes the water directly behind the fish. The high-speed camera system will record fish burst swimming motions as it swims to the opposite end of the raceway, at 500 frames/s. The high-speed video recordings will be analyzed image-by-image (Peak Performance Technologies, Inc., Centennial, Colorado) to determine velocities and acceleration rates

at specific distances, and fast-start body orientation (C-shape). Maximum burst swimming velocity will be determined as the greatest distance moved over a specified elapsed time (cm/s). Acceleration will be calculated as increasing velocity up to maximum burst swimming speed (m/s^2). The software automatically calibrates the pixels/cm with the filming information (resolution, recording speed) and the fish can then be tracked by two points on a centimeter grid. For determining C-start angles, we will compare a video segment before the C-start preparation stage, where the fish is mostly straight, to when it contracts and bends into a "C" shape to establish three points to measure contraction angles. Angle theta (θ) will be determined to be the angle made from the two intersecting lines meeting at the center of mass. Theta (θ) is recorded as the minimum angle when <180º and as the minimum explementary angle when >180º. Using the equation 0.35+ (0.2TL), where TL is the total length (mm) of a salmonid to determine the center of mass, we will manually track the trailing edge of the caudal fin, head, and center of mass points for each fish image-by-image. Weights (±0.01 g) and measurements (TL, ±1 mm) will be recorded for each fish using an electronic balance and measuring board.

168-Hour Survival Monitoring

Survival will be determined over a 168-h holding in 190-L tanks with either raw Delta or ozonated/ultraviolet sterilized water for control and each treatment group. Tanks will be examined daily for mortalities and those fish carefully removed so water quality is not degradated. After 168h, surviving fish will be counted, weighed (±0.01 g), and measured (TL, ±1 mm) using an electronic balance and fish measuring board.

Data Analyses

Statistical analyses will be performed using Sigmastat 3.0 (Jandel Scientific, San Rafael, California) software package. Differences between treatments and controls were tested using a factorial random complete block design (RCBD) analysis of variance (ANOVA; Zar 1984, Steel *et al.* 1997). The Tukey's test will be used for all pair-wise multiple comparisons for parametric data. The Shapiro-Wilk's test for normality and the Levene's test for homogeneity of variances will be used to determine ANOVA assumptions. Data that does not meet the ANOVA assumptions and is unable to be power or log transformed will be compared with a Kruskal-Wallis non-parametric ANOVA on ranks with the Dunn's test for pairwise multiple comparisons (Zar 1984, Steel *et al.* 1997). Differences will be considered significant at $P< 0.05$.

Coordination and Collaboration

This research will be a collaborative effort between Fisheries and Wildlife Research Group staff, Tracy Fish Collection Facility biologists and diversion workers. Research will be coordinated directly with the Tracy Technical Advisory Team, Tracy Fish Facility Improvement Program manager and the Tracy Fish Collection staff.

Endangered Species Concerns

This study will not involve the use of wild endangered or threatened species. Chinook salmon will be obtained from the Mokelumne River Hatchery (Clements, CA)

or Coleman National Fish Hatchery (Anderson, California). Applicable state and federal permits will be (have been) obtained to conduct research with this species.

Dissemination of Results (Deliverables and Outcomes)

The primary deliverable will be articles published in both the Tracy Volume Series and a peer-reviewed scientific journal. Technical updates will also be provided to the Tracy Technical Advisory Team and the Central Valley Fish Facilities review Team, along with an oral presentation given at a scientific forum in July 2010. Additional information will be supplied to National Marine Fisheries Service and the U.S. Fish and Wildlife service for reevaluating their Chinook salmon tissue sampling protocol.

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